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LINGO2 expression on T cells promotes anti-helminth immunity

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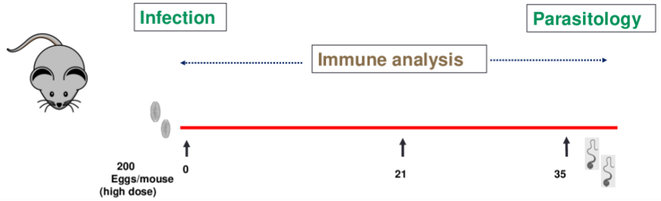
PURPOSE / OBJECTIVES

Leucine rich repeat and Ig domain containing 2 (LINGO2) is expressed in T cells but it has not been previously studied whether it plays a role in the differentiation and function of CD4⁺ T cells. We *hypothesize* that LINGO2 regulates CD4⁺ T cell biology and thereby governs the outcome of parasitic infection.

MATERIAL & METHODS

In this study we utilized the mouse model of the intestinal whipworm, *Trichuris muris* infection to probe the role of LINGO2 in CD4⁺ T cells. In this system, inoculation of wild type mice with a high dose of eggs result in the migration of larvae to the cecum and proximal colon which cause colitis and damage. T_H2 expansion and production of type 2 cytokines increase from 14 d post infection and peak at 21 d post infection resulting in expulsion of the worm infection from the intestines by 35 d post infection[4] and intestinal mucosal healing.



RESULTS

LINGO2 expression in T cells is necessary for parasite clearance and recovery from pathogen induced colitis

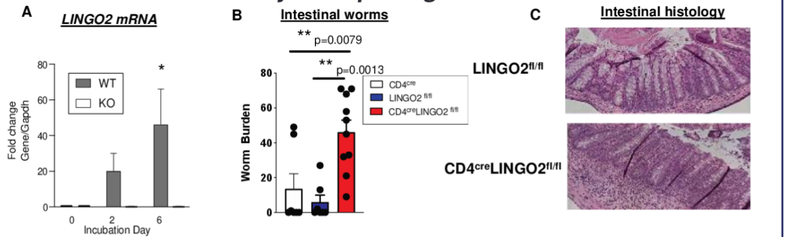


Fig 1. Selective LINGO2 deficiency in CD4⁺cells impairs control of *Trichuris muris* infection. (A) mRNA expression levels of *Lingo2* in IL2 and anti- CD3/CD28 Ab treated CD4⁺ T cells. **(B)** Worm burden of control versus CD4cre⁺LINGO2flox/flox mice 35 d post infection. Means of 7-8 mice/group and results of unpaired t test shown. **(C)** Representative H&E stained sections of the cecum of control versus CD4cre⁺LINGO2flox/flox mice at 35 d post infection.

ABSTRACT

T helper type 2 (T_H2) cells are critical for clearance of parasitic helminth infections. Their coordinated efforts harness cellular and molecular pathways to expel worms, resolve intestinal inflammation and drive tissue repair. Yet the mechanisms that control T_H2 cell activation, differentiation, and maintenance remain unknown. We recently demonstrated that Leucine rich repeat and Ig domain containing 2 (LINGO2) can function as a receptor for the mucosal reparative cytokine Trefoil factor 3 to limit epithelial damage, colitis and immunopathology. Because LINGO2 is broadly expressed in both hematopoietic and non-hematopoietic cell lineages, this study specifically addressed whether LINGO2 expression in the T cell compartment served a biologically important function in host protection against the parasitic helminth *Trichuris muris* . Data show that LINGO2 is upregulated in activated T cells and mice selectively deficient for LINGO2 in T lymphocytes (CD4Cre LINGO2$^{flox/flox}$) had significantly higher numbers of adult worms in the cecum, parasite eggs in the stool and colon immunopathology compared to CD4Cre or LINGO2$^{flox/flox}$ controls. Intriguingly, after *Trichuris infection,* CD4Cre LINGO2$^{flox/flox}$ mice had significantly fewer GATA3⁺ and IL4⁺ T_H2 cells in the mesenteric lymph node but significantly increased interferon gamma levels. Taken together, this work highlights a previously unrecognized cell-intrinsic role for LINGO2 in controlling T cell responses that critically shapes the outcome of helminth infection.

RESULTS

LINGO2 deficient MLN cells have decreased type 2 cytokine production

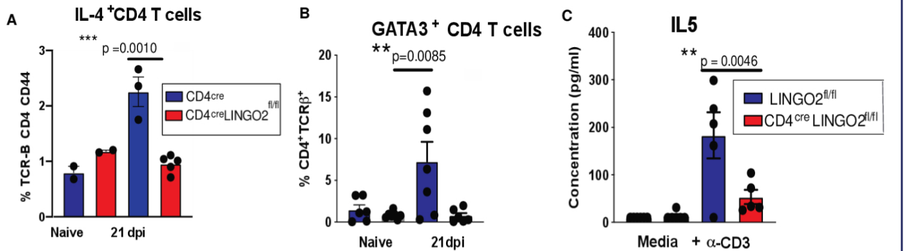


Fig 2. Mice deficient in LINGO2 in CD4⁺T cells and infected with *Trichuris muris* have less IL4 and GATA3 expressing cells in the MLN and produce less ex vivo IL5 at day 21. Quantification of (A) IL4+ and (B) GATA3⁺ T_H2 MLN cells in naïve and infected mice 21 days post infection. (**C**). IL5 protein expression by cytokine bead assay in MLN derived cells after 72 hrs of ex vivo stimulation with antI- CD3/CD28 Ab. Results of ANOVA shown.

RESULTS

Blockade of inappropriate IFN gamma production by LINGO2 deficient CD4+ T cells restores parasite clearance

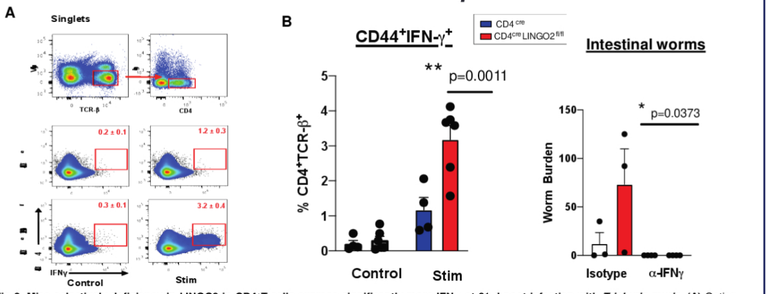


Fig 3. Mice selectively deficiency in LINGO2 in CD4⁺T cells express significantly more IFN-γ at 21 d post infection with *Trichuris muris*. (A) Gating strategy to identify CD4⁺TCR-β⁺CD44⁺from MLN that express IFN-γ at 21 d post infection. (**B**) Quantification of cells expressing IFN-γ at 21 d post infection. Means of 6 mice/group shown with analysis by two way ANOVA. (C) Worm burden of control versus CD4cre⁺LINGO2flox/flox mice at 35 d post infection. Mice were treated with either isotype Ab or anti-IFN-γ on days -1, 7, 14, 21 d post infection. Analysis by one way ANOVA.

SUMMARY / CONCLUSION

- **Mice selectively deficient in LINGO2 in CD4⁺T cells are unable to clear *Trichuris muris* infection compared to controls**
- **CD4⁺T cells from the MLN of selectively LINGO2 deficient mice inappropriately express IFN-gamma at 21 d post infection compared to controls. Blockade of IFN-gamma promotes worm clearance**

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